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NUTRITION REVIEWS

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ERRATUM

Page 171 (June issue) Column 1 Lines 6, 7, and 16

The word microgram should read milligram

NUTRITION REVIEWS

VOL. 5 JULY, 1947 No. 7

NUTRITION IN HEALTH EDUCATION

The nutritionist in health education has many important responsibilities. These range from an interpretation of the elementary facts of nutrition to problems dealing with international food and economic policies.

The nutritionist should help dispel the bewilderment of many people caused by overemphasis on fragmentary details concerning specific food factors, and insufficient attention to the over-all value of complete nutrition for total health. Overemphasis of a particular vitamin, of one source of iron or another, or of some special packaged form of protein, has given many persons a distorted view of nutritional values. It is the responsibility of the nutritionist in the field of health education to inform the public properly of food as related to total health.

Eating should be a healthful pleasure, not a laboratory experience. It is not necessary for every American to know how many micrograms of vitamin A are contained in his breakfast. The laboratory investigator must continue to expand detailed knowledge, but when the nutritionist presents these findings to the teacher, the nurse, or the general public the details ought to be placed in their true perspective in conjunction with other facts. The knowledge that is conveyed to the ultimate consumer should be dealt with as food values rather than as items in the pharmacopeia.

Civilization has contributed greatly to the cultural advancement of many peoples, but it also has introduced changes of another character which have proved detrimental to their physical well-being. Too often faulty changes in food habits accompany the acquirement of literacy and other refinements in a nation's way of living. When white rice was introduced to the Orient, the populations there were deprived of vital food substances contained in the unrefined rice to which they had grown accustomed as the basic product in their diets. Certain Indian tribes of Canada may have benefited from the cultural point of view when the white man interfered with their habits to raise their standards of living, but they suffered nutritional loss when he took away many of the natural foods and the tonics they had concocted from a variety of herbs that were rich in vitamins. Civilization, then, has a responsibility to see that proper nourishment is provided when it undertakes to alter any people's way of living.

World leaders are now beginning to realize that people are rationed by economic factors and agricultural limitations as well as by an understanding of their needs. They are also recognizing that food intakes often reflect external circumstances rather than fundamental needs and that some of these external circumstances are man-made and can be man-controlled. Consequently, the nutritionist in health education will find his ability to interpret nutritional needs limited by his own understanding of the several factors which have established the food habits prevalent in a particular family or community. The nutritionist in health education is participating not only in an intellectual discipline but also in a social activity, fully realizing that in addition to ignorance there are prejudice, poverty, agricultural practices, and economic policies to play a part in the total picture of malnutrition.

As education influences individuals and groups to an awareness of the part that good nutrition plays in total health, demands will

increase for an economy that will make possible adequate food for everyone. As social pressures demand higher nutritional levels of population groups, improved agricultural practices and altered economic policies should follow to make educational factors more effective.

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PRESENT KNOWLEDGE OF VITAMIN-ENZYME RELATIONSHIPS

In the earlier phases of nutrition studies, vitamins were necessarily regarded as rather mysterious food elements which in trace quantities served some essential catalytic role in the body. In a parallel field of biochemistry, that of enzyme chemistry, enzymes were also regarded in a similar way. During the rapid development of the latter field, certain enzymes were found to require coenzymes or "prosthetic groups," and the latter were often revealed to contain components later recognized as vitamins. In more recent years the discovery of new vitamins and their chemical structure has generally preceded the recognition of their function in metabolism. Certainly the knowledge of a given vitamin is incomplete without an understanding of its mechanistic role in metabolism, and such studies now constitute an important segment in the fields of both nutrition and biochemistry. Thus-far several of the B-complex vitamins have been recognized as components of enzyme systems, but definitive biochemical roles for many of the other vitamins have not been found.

The recognition of the function of a vitamin offers a basic approach in studies of the detection of nutritional deficiencies. In testing for the sufficiency of a given vitamin, the actual metabolic reaction involving the vitamin might be studied rather than measuring the amount of the vitamin in the blood or tissues. The latter is particularly subject to effects of recent vitamin intake and to interpretation of the significance of these levels to health. Unfortunately the former principle is difficult of application because of the complexities of the chemical reactions usually involved, and because the ideal test would seem to require tissue biopsy samples. Nevertheless the functioning of thiamine has been revealed by measurements of blood pyruvate, and the general principle would seem to be worthy of further study.

Thiamine. Perhaps the earliest recognized metabolic change dependent on a vitamin deficiency was the observation of N. Gavrilescu and R. A. Peters (*Biochem. J.* **25,** *1397 (1931)*) that the oxygen uptake of thiamine deficient pigeon brain was lowered, and later it was determined that both oxygen uptake and pyruvate removal were accelerated by addition of a thiamine concentrate to such a tissue preparation. The enzyme carboxylase was recognized in 1911 and the need of a coenzyme, cocarboxylase, in 1932. K. Lohmann and Ph. Schuster in 1937 (*Biochem. Z.* **294,** *188 (1937)*) found that cocarboxylase was the pyrophosphoric acid of thiamine. The coenzyme is now alternatively called cocarboxylase, thiamine pyrophosphate, or diphosphothiamine. It apparently acts in conjunction with several enzymes involved in the metabolism of pyruvate, although it is not known whether the coenzyme itself undergoes a change during its action.

Cocarboxylase can be determined chemically by modifications of the methods commonly applied to thiamine, and by the measurement of carbon dioxide produced during decarboxylation of pyruvic acid by washed yeast preparations. Cocarboxylase is lowered in several tissues in thiamine

deficiency, and the decrease in blood (*Nutrition Reviews* **3,** *93* (*1945*)), particularly in the leucocytes (*Ibid.* **1,** *84* (*1943*)), may offer a test for thiamine deficiency. The decrease in tissue cocarboxylase results in an increase in tissue and blood pyruvate. The increased blood pyruvate in polyneuritis was early recognized (R. H. S. Thompson and R. E. Johnson, *Biochem. J.* **29,** *694* (*1935*)) and has been amply confirmed. Unfortunately the blood pyruvate level may be increased by other factors (*Nutrition Reviews* **1,** *148* (*1943*); **2,** *305* (*1944*)) but with adequate control may become the most direct evaluation of the sufficiency of thiamine nutrition.

Riboflavin. In 1932, O. Warburg and W. Christian (*Naturwiss.* **20,** *688, 980* (*1932*)) announced the isolation of a "yellow enzyme" which in conjunction with a protein and a coenzyme (now recognized as triphosphopyridine nucleotide) cause the oxidation of glucose monophosphate. This yellow protein was split to produce a flavin, and the properties of "lumiflavin" were described. Shortly thereafter R. Kuhn, P. György, and T. Wagner-Jauregg (*Ber. d. deutsch. chem. Gesellsch.* **66,** *317* (*1933*)) isolated crystalline lactoflavin from milk, studied the lumiflavin derived from it, and identified it as a component of the vitamin B-complex which had growth promoting properties in rats on an otherwise flavin-free diet. Since these initial investigations several flavoproteins have been isolated and described, many of which serve as "mediators" for the transfer of electrons from pyridine nucleotides (coenzyme I and II) to oxygen or cytochrome. Others, such as xanthine oxidase and d-amino acid oxidase, serve as direct oxidative enzymes for specific metabolites. Riboflavin is itself a ribose-isoalloxazine; the flavoproteins contain as a prosthetic group either the mononucleotide riboflavin phosphate or the dinucleotide containing adenine as the second nitrogen base.

Although decreases in the tissue concentration of certain flavoproteins have been found in riboflavin deficiency (*Nutrition Reviews* **3,** *291* (*1945*)), no test on this basis has been suggested for detection of a dietary deficiency of this vitamin. The metabolic functions of riboflavin may be too varied to provide a single useful test, and it would not be improbable that the different biochemical functions show different degrees of sensitivity to riboflavin deficiency.

Niacin. A coenzyme for alcoholic fermentation was recognized in 1906 by A. Harden and W. J. Young (*Proc. Roy. Soc. (London)* **B77,** *405* (*1906*)), and this was isolated and characterized in a series of investigations by H. von Euler and associates during the years 1921 to 1934. A second coenzyme that is necessary for the oxidation of hexosemonophosphate was discovered by O. Warburg and W. Christian (*Biochem. Z.* **254,** *438* (*1932*)), and later found to contain nicotinamide as a component. The same was found true of von Euler's coenzyme. The latter is generally referred to as coenzyme I (or diphosphopyridine nucleotide) and the former as coenzyme II (or triphosphopyridine nucleotide). Both coenzymes function in transferring electrons from a variety of metabolites to flavoproteins, the niacin portion undergoing oxidation and reduction.

Although niacin was known chemically for many years, it was only in 1937 that C. A. Elvehjem, R. J. Madden, F. M. Strong, and D. W. Woolley (*J. Am. Chem. Soc.* **59,** *1767* (*1937*)) recognized it as a specific cure for blacktongue.

No tests based on metabolic function have been suggested to detect niacin deficiency. The rate of oxidation of a variety of metabolites which depend on coenzymes I and II might be utilized, but in each case the oxidation would also be dependent on flavoproteins and possibly several other variable factors. A decrease in the coenzyme I content of heart muscle has been reported during experimental anoxia (*Nutrition Reviews* **3,** *236* (*1945*)).

Pyridoxine. During the years 1942 to 1944

several workers noted the abnormal excretion of kynurenine and xanthurenic acid in pyridoxine deficiency (*Nutrition Reviews* **3,** *72* (*1945*)). These products apparently resulted from an abnormal metabolism of tryptophane, and disappeared upon correcting the pyridoxine deficiency. Also in 1944 I. C. Gunsalus, W. D. Bellamy, and W. W. Umbreit (*J. Biol. Chem.* **155,** *685* (*1944*)) found that pyridoxal phosphate was a coenzyme in the decarboxylation of tyrosine by certain bacteria, and E. F. Gale and H. M. R. Epps (*Biochem. J.* **38,** *232*, *250* (*1944*)) isolated a natural coenzyme from yeast which contained a pyridoxine component and acted as a lysine codecarboxylase. This subject was developed rapidly, particularly by Gunsalus and his co-workers at Cornell, who showed that pyridoxal phosphate functioned also as a coenzyme for transaminase. The transaminase activity of tissues has been reported to be low in pyridoxine deficiency (F. Schlenk and E. E. Snell, *J. Biol. Chem.* **157,** *425* (*1945*)).

Rigorous proof for the chemical structure of pyridoxal phosphate is lacking, and the precise mode of action of the coenzyme during the decarboxylation and transamination functions is not clear. The recognition of pyridoxal phosphate as codecarboxylase and cotransaminase has been developed in greater detail in separate reviews (*Nutrition Reviews* **3,** *63*, *72*, *343* (*1945*); **4,** *232* (*1946*)).

Pantothenic Acid. It has been recognized for some time that the pantothenic acid in blood is principally in the bound form (L. D. Wright, *J. Biol. Chem.* **147,** *261* (*1943*)), a situation which might be expected if the vitamin were associated with a protein as a coenzyme. It has also been reported that this bound form is decreased after glucose administration, indicating its possible participation in glucose metabolism, and that pyruvate oxidation is decreased in pantothenic acid deficient bacteria or liver (*Nutrition Reviews* **2,** *21* (*1944*)). Only recently have F. Lipmann and co-workers (*J. Biol. Chem.* **167,** *869* (*1947*)) demonstrated a definitive role for pantothenic acid as a component of the coenzyme for acetylation. Pantothenic acid is therefore concerned with biologic acetylations such as the formation of acetylcholine or the detoxification of certain drugs.

Biotin. In studies of the oxidation of lactate and pyruvate by liver tissue of biotin deficient rats, W. H. Summerson, J. M. Lee, and C. W. H. Partridge (*Science* **100,** *250* (*1944*)) indicate that biotin may be involved in the oxidation of these metabolites. In view of the complexity of pyruvate metabolism, there are many possibilities for a more specific function.

Para-Aminobenzoic Acid and Folic Acid. PAB has been found to occur in a bound form, either as a peptide with glutamic acid (*Nutrition Reviews* **3,** *108* (*1945*); **5,** *31* (*1947*)) or as a component of folic acid *Ibid.* **4,** *199* (*1946*)). Despite recent knowledge of the chemical structure of folic acid and its use in the macrocytic anemias, J. E. Davis (*Science* **104,** *37*, *605* (*1946*)) has already suggested a biochemical function of folic acid in the nature of a control of serum cholinesterase and acetylcholine levels.

Ascorbic Acid. The oxidation-reduction properties of ascorbic acid are well known, yet there is little evidence that this vitamin serves as a redox catalyst in tissue metabolism (*Nutrition Reviews* **2,** *283* (*1944*)). Possibly the most promising lead as to a specific biochemical function of ascorbic acid is that of T. Ho Lan and R. R. Sealock (*J. Biol. Chem.* **155,** *483* (*1944*)) who demonstrated an incomplete metabolism of tyrosine and phenylalanine in ascorbic acid deficiency.

Other Vitamins. Less specific suggestions can be made for the biochemical function of the remaining vitamins. Vitamin E appears to act principally as a physiologic antioxidant, although the reported increase of oxygen consumption and succinoxidase activity of vitamin E deficient muscle may suggest a specific role in tissue respiration processes (*Nutrition Reviews* **1,** *308* (*1943*)). Nothing specific can be suggested as to the mode of action of vitamins A or D.

It is strange that with the increasing numbers and study of vitamin inhibitors (analogues) or antimetabolites, it is not yet clear whether these act merely by preventing the biologic synthesis of vitamins or whether they might inhibit the physiologic action of preformed vitamins. The action of these substances on enzyme systems *in vitro* has not been reported. Since vitamin analogues are available for vitamins whose specific functions are as yet unknown, the application of these new tools may accelerate our knowledge of vitamin-enzyme relationships.

THE EFFECT OF CORN MEAL ON RATS RECEIVING LOW PROTEIN DIETS

The replacement by corn products of part of a low casein synthetic ration devoid of niacin depresses the growth rate of rats maintained on the ration (*Nutrition Reviews* **4,** *334* (*1946*)). When the diet contains an ample supply of protein (or of the specific amino acid tryptophane) or of niacin, then the introduction of corn products does not affect the growth unfavorably.

This phenomenon has been further investigated by H. Spector and H. H. Mitchell (*J. Biol. Chem.* **165,** *37* (*1946*)), who remarked that since 1914 various investigators have been led to the conclusion that deficiency of one or more essential amino acids may be a factor in the etiology of pellagra. J. Goldberger and W. F. Tanner (*Pub. Health Reports* **37,** *462* (*1922*)) observed that some beneficial results in the treatment of pellagrins were obtained with tryptophane and cystine. A related effect was also reported by H. Chick and E. M. Hume (*Biochem. J.* **14,** *125* (*1920*)) who produced symptoms in monkeys which closely resembled those of pellagra by prolonged restriction to a low protein diet. The treatment of one monkey with tryptophane served to prolong its life by many weeks.

H. Spector and H. H. Mitchell (*loc. cit.*) point out the possibility that "a depression of appetite may account for the effect of corn in depressing growth and its counteraction by niacin or tryptophane," and they question to what extent the growth retardation is due to a specific vitamin (or amino acid) deficiency and to what extent it is due merely to generalized undernutrition. The answer to this question might be obtained by use of the paired feeding technic in observing the effect of corn on rats maintained on a niacin-free, low casein diet. The authors therefore carried out such experiments, and repeated the ad libitum feeding experiments previously reported by others.

The results of the ad libitum feeding tests showed that the inclusion of corn grits in the basal synthetic ration caused a marked depression of growth associated with a decreased food consumption. The further addition of niacin or tryptophane to the ration produced a marked improvement in food consumption and weight gain. The paired feeding trials showed that the inclusion of niacin or tryptophane in the diet containing corn grits produced a small but significant increase in the efficiency of utilization of the food. They showed further that most of the stimulation in growth produced by adding niacin or tryptophane to the diet containing corn grits was due to the associated increases in caloric intake. When food intakes were equalized, the growth stimulation due to niacin or tryptophane supplements was reduced from the 16 g. per week observed with ad libitum feeding to only 1 g. per week.

The original depression in growth due to including corn grits in the diet was similarly decreased from 16.7 g. weekly to 2.6 g. weekly by equalizing the food intakes of corresponding rats. From these observations, together with others recorded in the literature, the authors concluded that the physiologic effects of niacin and tryptophane

are not related to a modification in the synthetic capacities of the body or of the microorganisms of the intestinal tract. Niacin and tryptophane nevertheless participate interchangeably, and the authors suggest the relation of choline and methionine as illustrative of the type of interchange which may occur.

MICRODETERMINATION OF CAROTENE AND VITAMIN A IN NUTRITION SURVEYS

The development of rapid and convenient methods for the accurate determination of nutrients or their derivatives is of the greatest significance for their application in nutrition survey work (*Nutrition Reviews* **3,** *336* (*1945*)). Another in the series of methods developed by O. H. Lowry and O. A. Bessey and their co-workers at the Public Health Research Institute of the City of New York has now been described; it deals with the estimation of vitamin A and carotene in small quantities of blood serum (O. A. Bessey, O. H. Lowry, M. J. Brock, and J. A. Lopez, *J. Biol. Chem.* **166,** *177* (*1946*)).

In choosing the type of method to be used, the authors initially considered and rejected any method based on the Carr-Price test, since the volatility of the solvents employed would render any application of the micro scale very difficult, as slight evaporation of the solvents would almost certainly lead to the condensation of water vapor in the solution with consequent appearance of unspecific turbidity due to the formation of antimony oxychloride. The method chosen was therefore of necessity based on the measurement of the ultraviolet absorption of vitamin A at 328 millimicrons, although the optical density at that wavelength is only about one-third as great as that of the blue color produced with the Carr-Price reagent at 620 millimicrons.

The difficulty in using the measurement at 328 millimicrons is, however, due less to the smaller absorption at that wavelength than to the absorption of other substances in addition to vitamin A. The principle of the method chosen to eliminate the absorption of other compounds was used by R. W. Little (*Ind. Eng. Chem. (Anal. Ed.)* **16,** *288* (*1944*)). In measuring the vitamin A potency of fish liver oils containing relatively little vitamin A he measured the absorption before and after irradiation of the sample solution of oil with ultraviolet rays of wavelengths of 310 to 400 millimicrons. This type of method had indeed previously been applied to blood serum on the macro scale by A. Chevallier, Y. Choron, and R. Matheron (*Compt. rend. Soc. biol.* **127,** *541* (*1938*)), although they did not fully explore the possibilities of this technic.

The method as finally devised consisted first in saponifying the vitamin A of the serum by heating with alcoholic potash, and then extracting it from the aqueous phase into a nonvolatile mixture of equal parts of xylene and kerosene. A determination is made on only 60 cu. mm. of serum, and conditions are described to treat up to one hundred tubes at the same time. Extraction is accomplished by holding the tube at a 45° angle against a rapidly spinning flattened nail for ten to fifteen seconds. Separation of the layers is effected by centrifugation, the kerosene-xylene layer pipetted into the special narrow Beckman cuvette (*Nutrition Reviews* **5,** *71* (*1947*)), and the transmission read at 460 and 328 millimicrons. The samples are then transferred to micro test tubes for irradiation with a suitable ultraviolet lamp, and a second reading taken at 328 millimicrons. Special precautions, such as rinsing the transfer pipet with anhydrous propionic acid, are taken against the possibility of turbidity due to traces of moisture.

The reliability and precision of the new procedure were studied extensively by the authors, with results which enabled them to place complete confidence in their method. It was a major problem to find a solvent which would completely extract vitamin A and carotene from serum and permit the manipulation of small volumes without evaporation. Petroleum ether, which is used in macro scale work, was unsuited because of its volatility; toluene was an improvement, and xylene was still better. Kerosene showed little or no evaporation, but failed to extract the vitamin A and carotene quantitatively. A mixture of equal volumes of kerosene and xylene was found to give negligible evaporation, while recovery experiments from serum showed complete recovery of both pigments. For purposes of calculating the factor for conversion of the ΔE_{328} reading to micrograms of vitamin A it was necessary to determine the details of the vitamin A spectrum in this mixture in which vitamin A ester has only 96 per cent as much absorption as in alcohol, but retains 3 per cent as much after destruction by prolonged irradiation. Similarly it was necessary to determine the absorption coefficient of carotene in xylene-kerosene.

By plotting the difference-spectrum between 310 millimicrons and 390 millimicrons and showing its near identity with the difference-spectrum obtained with pure vitamin A, it was shown that the substance in the serum extract being measured by the difference-absorption was indeed vitamin A. The new micro method yielded somewhat lower values than the Carr-Price procedure with a series of serums, and evidence is presented to suggest that this is due to the known inhibitory effect of interfering substances on the latter reaction. The use of an "internal standard" for the Carr-Price reaction resulted in close agreement between the results of the two methods.

Finally, since it is convenient to collect a batch of samples in the field and then take them to a central laboratory for analysis, the keeping quality of the serum samples was studied. Samples of two serums were stored in sealed capillary tubes and analyzed after one and four and one-half months. There was no loss of vitamin A or carotene after one month at either 4° or −20°. After four and one-half months there was no loss of vitamin A and a 6 per cent loss of carotene at −20°; at 4° the carotene was almost completely destroyed, and the vitamin A was unchanged in one sample, while 60 per cent was lost from another. It seems that serums can be safely stored for several weeks at 4° or several months at −20° without loss. The method can be considered conveniently rapid for large-scale survey work or for experiments on small animals because one analyst can carry out fifty determinations in a working day.

CADMIUM AND DENTAL CARIES SUSCEPTIBILITY

Cadmium poisoning has frequently been encountered in man after the ingestion of mildly acid beverages prepared in vessels plated with cadmium which is readily dissolved by weak acids (*Nutrition Reviews* **3,** *156* (*1946*)). Few studies of cadmium toxicity in experimental animals have been made. R. H. Wilson, F. DeEds, and A. J. Cox (*J. Pharmacol. Exp. Therap.* **71,** *222* (*1941*)) noted that one of the first signs of cadmium poisoning of a chronic type in the white rat was a decrease in the deposition of the normal orange-brown pigment in the labial enamel of the incisor, similar to the inhibition of pigment formation observed in chronic fluorine toxicity (*Nutrition Reviews* **4,** *227* (*1946*)).

The pigment in the rat incisor has been demonstrated by numerous investigators to contain iron; not all of the iron in the incisor is contained in this pigment (E. V. Pindborg, J. J. Pindborg, and C. M. Plum,

Acta Pharmacol. Toxicol. **2,** *285* (*1946*)). These workers studied the effect of an iron deficiency on the pigmentation of the rat incisor. An iron deficiency was produced by the removal of 2 ml. of blood from each rat every second day throughout the experiment. The removal of this amount of blood was reflected by a reduced hemoglobin concentration, a low erythrocyte count, and by a reduction of the liver iron by 50 per cent. The iron content of the incisors was reduced about 30 per cent but there was little effect upon the deposition of pigment in the incisors. The addition of iron to the stock ration during the experimental period reduced the decrease of iron in the incisors but had little effect on the iron content of the liver. In studies on the effect of cadmium poisoning on iron metabolism, these same investigators (*Ibid.* **2,** *302* (*1946*)) noted a decrease in the iron content of the liver, an anemia with a considerable increase in reticulocytes, and a decrease in the pigment deposition in the incisor accompanying the generalized alteration in iron metabolism. In fluorine toxicity, they observed abnormal iron metabolism with a decrease in the iron content of the liver and the teeth, an anemia with no increase in reticulocyte count, and a decrease in pigment deposition in the incisors (*Ibid.* **2,** *294* (*1946*)). This series of studies indicates that some of the effects of cadmium and fluorine toxicity are somewhat similar and are manifest through derangement in the iron metabolism. In this particular case the toxicologic effect of cadmium and fluorine on iron metabolism was greater than that produced by severe bleeding at frequent intervals. Since a stock ration containing some iron was used in the iron deficiency studies, the severity of the iron deficiency produced may have been less than if a stringently iron deficient ration had been used.

B. F. Miller (*Proc. Soc. Exp. Biol. Med.* **39,** *389* (*1938*)) and other investigators have observed an inhibiting effect of relatively high amounts of fluorine in the ration or in the drinking water upon the incidence of carious lesions in white rats. Since cadmium poisoning produces similar gross signs, it was of interest to study the effect of cadmium on the incidence of tooth decay. J. T. Ginn and J. F. Volker (*Ibid.* **57,** *189* (*1944*)) fed rats a caries-producing ration which contained 0.005 per cent cadmium chloride and water which contained a similar amount. The cadmium in the food had no effect on the incidence and progress of carious lesions but that in the water produced an increase in the dental caries incidence and a more rapid development of the carious lesions. In a further exploration of this phenomenon, H. M. Leicester (*J. Dental Research* **25,** *337* (*1946*)) fed water containing either 0.002 or 0.004 per cent cadmium chloride and the Hoppert-Webber-Canniff coarse particle diet from weaning for one hundred days. The rats given drinking water with 0.004 per cent added cadmium chloride had the same average dental caries index as the controls and those receiving drinking water with 0.002 per cent of added cadmium chloride, but there was a wider range of caries index values among the rats in the higher cadmium chloride group. The difference was not as clearcut as that reported by Ginn and Volker.

To determine whether the effect of cadmium could be altered if the cadmium salt were administered during the period in which the teeth are being differentiated and calcified, Leicester (*loc. cit.*) began to feed pregnant rats drinking water containing 0.004 per cent cadmium chloride two to six days before parturition. Half of the offspring were continued on this supplement until one hundred days after weaning (at twenty-three days) when the molar crowns were completely calcified; the other half were given tap water from weaning until the end of the experiment. No appreciable difference was observed between the average dental caries index of the offspring of control rats which received no cadmium chloride throughout life and the average dental

caries index of the rats which had had a supplement of cadmium chloride during tooth formation. The rats which received cadmium chloride during the period of tooth development and posteruptively had an average dental caries index about double that of the other two groups. Thus the effect of cadmium on dental caries appears to be opposite from that of fluorine, in that (1) cadmium stimulates the production of carious lesions in some manner, while enough fluorine inhibits the carious process, and (2) cadmium's effect is greatest post-developmentally, while the evidence indicates both for man and experimental animals, that the effect of fluorine-containing waters is greatest during tooth development.

Cadmium is the first element known to have been described as possessing a stimulatory effect on dental caries activity in experimental animals. It is unknown at this time whether cadmium or other elements or unrelated compounds with similar stimulatory properties introduced accidentally into the human dietary in greater than physiologic amounts might have a stimulating effect on dental caries activity in man. One suggestion of this type has been made by H. Klein (*Science* **105,** *4 (1947)*) as a result of a survey of the dental caries experience of migrants into regions with and without fluorine in the drinking water.

Approximately 3000 children were examined in five southern New Jersey communities, and the results analyzed for 1307 persons of 5 to 19 years of age who had come to these communities during the various times previous to observation. In three of these communities the drinking water contained 1.4 to 2.2 parts per million of fluorine, and in the other two the water was essentially fluoride-free. A total of 882 subjects had migrated into the "fluorine communities" and 425 into the "nonfluorine communities." The data were broken down into the 5 to 9, 10 to 14, and 15 to 19 year old age groups (at time of examinationd (an, these into 0 to 4, 5 to 9, and 10 to 14 years of exposure of the subjects to the two types of drinking water. The caries rate was expressed as the number of permanent (second) teeth decayed, missing, or filled per 100 person-years of age.

In each age group in the fluoride areas the caries rate decreased with increasing years of exposure to the drinking water. With a given duration of exposure, less protection against caries was evident in the older age groups than the younger. Part of this might have been due to previous years of the subjects in areas with less fluoride, but the difference in caries rate was evident in the groups of 10 to 14 years exposure. In comparing the fluoride and nonfluoride areas, the expected difference in caries rate was evident in each age group and in each exposure time with the exception of the two older groups with the 0 to 4 year exposure. It appears then that the fluoride protection is effective principally during the first decade of life. Whether the protection is entirely restricted to this period could only be determined by studying subjects migrating into fluoride areas at much later periods of life.

The investigator was interested principally in the increasing caries rate with duration of exposure in the nonfluoride areas. As examples, in the 10 to 14 year age group, the rate increased from 37.6 for the group with 0 to 4 years of exposure to 57.5 for 10 to 14 years of exposure, and in the 15 to 19 year age group from 49.6 to 70.0 over the same period. It is suggested therefore that just as fluoride favors *resistance* to tooth decay, "there may be factors in drinking water which make the teeth more vulnerable to dental caries." The nonfluoride water was found in preliminary investigation to be acid and high in iron and nitrate.

It is not clear from the data why a positive agent is the chosen alternative for the increased caries rate. As a corollary to the accepted action of fluoride in preventing caries, there seems no evident reason why continued exposure to lack of fluoride during tooth formation might not increase susceptibility to caries attack.

THE METHIONINE CONTENT OF MEAT

C. M. Lyman *et al.* (*J. Biol. Chem.* **166,** *161* (*1946*)) developed a microbiologic method for the assay of methionine. They attempted to supply most of the amino acids required in the assay medium by a relatively simple peptone preparation. Based on the preferential oxidation method of G. Toennies and T. P. Callan (*Ibid.* **129,** *481* (*1939*)), C. M. Lyman, O. Moseley, S. Wood, and F. Hale (*Arch. Biochem.* **10,** *427* (*1946*)) had developed a method for removing methionine, cystine, tryptophane, and tyrosine from peptone preparations by hydrogen peroxide. Such a preparation was a convenient nitrogen source for microbiologic tests.

In making a medium for methionine assay, the authors therefore used the hydrogen peroxide-treated peptone, with added tryptophane, tyrosine, cystine, adenine, guanine, uracil, vitamins, and salts. With this medium the organism Leuconostoc mesenteroides was used, and the method was tested in the usual ways for specificity and reproducibility. A second microbiologic method employing Streptococcus faecalis R, grown in a medium supplying nitrogen in the form of a mixture of pure amino acids was also developed, and the two methods were compared in the analysis of several proteins of widely varying methionine content. The two methods gave surprisingly close results. Both microbiologic methods were then compared further with a chemical method in the analysis of samples of beef loin, beef liver, and three different samples of casein. The results again showed extremely satisfactory agreement. The method using the medium containing hydrogen peroxide-treated peptone and L. mesenteroides was then applied to a variety of foodstuffs and the results tabulated.

In a second paper, the same authors (*J. Biol. Chem.* **166,** *173* (*1946*)) studied the methionine content of different meats, the variability in different samples of the same kind of meat, and the methionine content of corresponding organs and tissues from cattle, hogs, and sheep. The samples were ground and then dehydrated by a simple and ingenious procedure which the authors describe. The powdered dehydrated sample was used for the determination of nitrogen and of methionine. It was also ether-extracted, and when appreciable amounts of fat were present in the extract, correction for its nitrogen content was made in calculating the protein content of the meat. Fifty-six meat samples were assayed, including beef loin, round, brisket, liver, heart, kidney, tongue, sweetbreads, and spleen; pork loin, liver, heart, kidney, and tongue; and lamb chops, liver, kidney, and heart.

There was little variation in the methionine content of the same kind of meat taken from different carcasses, and the methionine content of any given tissue was nearly identical whether it came from beef, hog, or sheep. Beef brisket, loin, or round, and pork loin and lamb chops all contained about 0.50 per cent of methionine, while the liver and heart of all three species contained about 0.40 per cent, and the kidneys contained about 0.30 per cent. Beef brain, thymus, and spleen contained about 0.21, 0.22, and 0.36 per cent respectively. The amount of methionine in the protein of the samples varied from about 2.5 per cent for the muscle meats to 2 per cent for other organs, and as low as 1.4 per cent for the beef thymus.

METABOLISM OF ARGININE

The late Professor Schoenheimer and his colleagues established, by the use of isotopic tracer elements, that the proteins of the body are relatively rapidly "turned over" and that following absorption an amino acid interacts with the tissues by replacing the same acid in the proteins, and also yields up its nitrogen to build other constituents for protein synthesis by the processes of deamination, reamination, and transamination (R. Schoenheimer, "The Dynamic State of Body Constituents," *Harvard University Press* (*1942*)).

The alpha-amino groups of amino acids in general are reactive in this way. K. Bloch (*J. Biol. Chem.* **165,** *469* (*1946*)) has followed up the fate of the nitrogen of the amidine group in arginine. The rapid intermediary formation of this amidine group has already been shown by R. Schoenheimer, S. Ratner, and D. Rittenberg (*Ibid.* **130,** *703* (*1939*)), whose observations give support to the theory of the ornithine cycle proposed by H. A. Krebs and H. Henseleit (*Z. f. physiol. Chem.* **210,** *33* (*1932*)). Ornithine arising from arginine by arginase action enters into combination with carbon dioxide and ammonia or amino acid nitrogen to regenerate the amidine group, whose only known reactions are its participation in the formation of urea and its transfer to glycine in creatine formation.

Rats were therefore fed 1(+) arginine containing isotopic N^{15}, and the isotopic N^{15} was traced in excreta and in body constituents. The results showed that there is a rapid regeneration of the amidine group of arginine in the liver, in agreement with the theory of the ornithine cycle, and that the only other reaction of intermediary metabolism in which the amidine nitrogen of arginine takes part is the formation of creatine.

As mentioned by K. Bloch (*J. Biol. Chem.* **165,** *477* (*1946*)), arginine occupies a unique position among amino acids in mammals as a vehicle for nitrogen in the catabolism of proteins, because nitrogen arising from amino acid breakdown becomes incorporated into the amidine group of arginine, and the group is then split off as urea, leaving ornithine to go through the same cycle again. It is improbable that the arginine-ornithine cycle is of similar significance for birds because as an animal class they excrete nitrogen not as urea but as uric acid, and arginase (an essential enzyme for the ornithine cycle) is not present in avian livers. Also since ornithine cannot replace arginine which is indispensable in avian nutrition, it appears that birds cannot transform the former into the latter.

Similar experiments to those described above on rats were therefore made on pigeons (*loc. cit.*) and in this species the amidine group of arginine could not be synthesized at all, in contrast to the easy formation in the rat. In this way the difference between the alpha-amino nitrogen and the amidine nitrogen of arginine with regard to lability in the organism was strongly contrasted. In the pigeon, amidine nitrogen took part in no reactions, and in the rat it took part in only the two specific reactions of urea and creatine formation.

TOOTH DECAY IN THE HAMSTER

The Syrian hamster is susceptible to tooth decay under somewhat different circumstances from those of the white rat (*Nutrition Reviews* **1,** *205* (*1943*)). The chief differences are the greater freedom of the hamster from the association of fractures of the molar cusps with the development of carious lesions and the high susceptibility of

the hamster to tooth decay when fed diets composed of fine particles.

P. H. Keyes (*J. Dental Research* **25,** *341 (1946)*) made a systematic study of the types and distribution of carious lesions in the hamster when fed a diet consisting in per cent of: whole wheat flour 20, corn starch 25, confectioner's sugar 20, whole powdered milk 30, and alfalfa 5, plus carrots or fresh alfalfa once a week. One group of hamsters was not fed any supplement of "greens" without marked effect on the caries incidence. Gross evidence of carious lesions was commonly found in the molar teeth of hamsters which had been maintained on this diet for as little as twenty days. All animals killed after fifty days on experiment had carious lesions of the molar teeth and 91.6 per cent of the molar teeth of hamsters maintained on the diet for seventy-four to eighty-one days had carious lesions. The volume of tooth structure involved by the carious process increased directly with the duration of the experiment. Older animals were less susceptible to tooth decay but none was found to be immune to the carious process.

A considerable amount of a white, pasty material, resembling the so-called materia alba on human teeth, was frequently seen on the molars of the hamster after short periods on the cariogenic diet. No material of this type was found on the molars of animals maintained on the noncariogenic stock diet which was composed of Purina rabbit checkers and fresh green alfalfa. Within ten days after the animals were changed from this stock diet to the cariogenic diet, there were definite accumulations of this material; after thirty days, gross accumulations of the material covered large surfaces of the molar crowns, especially on the regions of highest caries susceptibility. Deposits of the material were found to be common subgingivally.

The carious lesions were of two general types: (1) subgingival or gingival crevice lesions, and (2) supragingival lesions. The former were observed below or at the free margin of the gums, usually on the buccal and lingual surfaces of the molars, but occasionally on the interproximal surfaces; the latter type of lesion was observed in the fossae on the occlusal surfaces, associated with the pasty, white deposits on the smooth, cusp surfaces, and as extensions of the subgingival lesions. In sections stained with Gram stain, the white material and the invaded areas were filled with masses of small round Gram-positive particles resembling microorganisms. Other bacterial forms were found in and around these carious areas, but the spherical types predominated.

With the indication that caries in the hamster resembles that in man, P. H. Keyes (*J. Nutrition* **32,** *525 (1946)*) started comparisons of the caries-producing properties of different rations. Four dietary regimens were studied: (1) a basal ration of whole yellow corn, (2) whole yellow corn ad libitum plus a supplement of whole powdered milk and dry powdered alfalfa, (3) the standard Hoppert-Webber-Canniff diet (which has been widely used for the production of tooth decay in the white rat through the mechanism of coarse corn particles), and (4) a ration composed of 2 parts of the Hoppert-Webber-Canniff ration and 1 part of confectioner's sugar. The growth rate of the animals on the Hoppert-Webber-Canniff ration was the highest, and those fed the corn diet the lowest; the incidence of caries in those fed the corn diet was extremely low. Inclusion of one-third sugar in the ration caused a great increase in dental caries activity with a greater number of teeth involved and a greater extent of cavitation. However, addition of sugar to the ration was made without compensation for the reduced amounts of other nutrients in the diet. The growth rate of these animals was lower than those without the sugar addition, hence there is indication that the sugar-supplemented ration was less adequate.

Since many difficulties are encountered in manipulating the composition of natural rations with respect to any one essential nutrient and still maintain desired physical characteristics of the ration, the next step would seem to be in the direction of purified rations. Such studies have been made with the cotton rat (*Nutrition Reviews* **3,** *83* (*1945*); **4,** *73, 308* (*1946*)); and the rhesus monkey (*Ibid.* **4,** *115* (*1946*)).

Extended studies with the hamster seem justified in that P. H. Keyes (*J. Dental Research* **25,** *341* (*1946*)) concludes that "the fundamental process of dental caries in hamsters appears to be very similar to that described in humans. Such differences as there are seem to be more of a quantitative than a qualitative nature." With the present data this hypothesis might be somewhat premature. Undoubtedly the type of lesion can be said to be more similar to human lesions than those in the white rat by reason of the freedom from a particle size requirement for the production of lesions in hamster molars. How similar the initiation and progress of lesions in the hamster are to those in man can only be determined by continued investigations of the numerous interrelated factors involved in the carious process in these two species. Keyes' data definitely indicate that the hamster is an animal which can be used for a wide variety of fundamental approaches to the dental caries problem because of its high susceptibility under a reasonably rigid dietary regimen.

FOLIC ACID AND NIACIN DEFICIENCY IN THE DOG

For a number of years there has existed suggestive, but inconclusive, evidence for the relationship of niacin and some hematopoietic factor in the dog. In 1933 C. P. Rhoads and D. K. Miller (*J. Exp. Med.* **58,** *585* (*1933*); *Proc. Soc. Exp. Biol. Med.* **30,** *540* (*1933*)) reported the irregular appearance of an anemia in dogs fed on a diet which produced chronic canine blacktongue. D. K. Miller and C. P. Rhoads (*Ibid.* **30,** *540* (*1933*); *J. Exp. Med.* **61,** *173* (*1935*)) also noted that a certain number of the dogs fed their blacktongue producing diet developed "an ulcerative stomatitis associated with leucopenia and granulopenia." Several years later P. L. Day and co-workers (*Ibid.* **72,** *463* (*1940*)) demonstrated that the widely used Goldberger blacktongue producing diet was deficient in vitamin M (folic acid) for the monkey. Although proof is not yet available that the above noted hematologic changes in the dog were due to the same deficiency as became manifest in the monkey, two papers from the Wisconsin laboratories strongly suggest that such may have been the case.

W. A. Krehl and C. A. Elvehjem (*J. Biol. Chem.* **158,** *173* (*1945*)) investigated the response of dogs to niacin when the animals were maintained on a niacin-low diet. The ration employed consisted of sucrose 66; cottonseed oil 11; acid-washed casein 19; and a high manganese salt mixture 4 parts. This basal diet was fed ad libitum, and in addition, each dog received 100 micrograms of riboflavin, 60 micrograms of pyridoxine hydrochloride, 500 micrograms of calcium pantothenate, and 50 mg. of choline chloride per kilogram of body weight per day, and 3 drops of halibut liver oil per kilogram of body weight per week. This ration contained 0.64 microgram of niacin per gram. It should be noted that this is not a Goldberger blacktongue producing diet; it contained no corn meal or black-eyed peas. Eighteen dogs were maintained on this basal ration; 10 of these received, in addition, a folic acid concentrate prepared from solubilized liver extract and administered in quantities of 1.5 ml. per dog per day. This amount contained the equivalent of 11.7

micrograms of vitamin Bc as measured by Streptococcus lactis assay.

After niacin deficiency developed in the dog, as indicated by drastic weight loss, anorexia, inflammation of the gums, and bloody diarrhea, the animals were given a standard amount of niacin. The weight responses to the administration of 25 mg. of niacin by 5 animals maintained on the basal diet alone ranged from 400 to 750 g. Similar responses to the same dose of niacin by the dog receiving the basal diet plus the folic acid concentrate ranged from 1000 to 1850 g. Following these initial responses to niacin the dogs which had not received folic acid lost weight rather rapidly and failed to respond a second time to administration of 25 mg. of niacin. By contrast, the dogs which had received folic acid, in addition to the synthetic basal ration, responded repeatedly with weight gains following the administration of niacin. Furthermore, those animals receiving folic acid did not exhibit the severe mouth lesions so characteristic of classic blacktongue. Finally, examination of the blood of these two groups of animals revealed that, despite considerable overlapping of values, there is a trend toward high hemoglobin levels and white cell counts in the dogs receiving the basal diet plus folic acid over the group of dogs receiving the basal diet alone.

In an extension of these studies W. A. Krehl, N. Torbet, J. de la Huerga, and C. A. Elvehjem (*Arch. Biochem.* **11,** *363 (1946)* employed the same basal diet but supplemented it with synthetic L. casei factor (pteroylglutamic acid). The pteroylglutamic acid was fed at a level of 25 micrograms per dog per day. Again, in these experiments in which the synthetic L. casei factor replaced the crude norit eluate used in the previously discussed experiments, the investigators were able to induce repeated growth responses to niacin. When the pteroylglutamic acid was then removed from the diet, the same dosage of niacin (25 mg.) failed to induce more than one additional growth response, and death ensued. The authors further noted that the anemia in the animals receiving the basal diet plus pteroylglutamic acid was less profound than in dogs receiving the niacin deficient diet alone. These studies, considered together with the recent demonstrations that pteroylglutamic acid is a member of the folic acid complex, indicate that the dog also requires this factor. These studies further suggest that the picture of blacktongue, observed on the Goldberger blacktongue producing diet, may be a complex one in which the symptoms of niacin deficiency predominate in some animals and those of folic acid deficiency predominate in others. The similarity of sprue to the syndrome in the latter type, noted by D. K. Miller and C. P. Rhoads (*Proc. Soc. Exp. Biol. Med.* **30,** *540 (1933)*), is now more evident since folic acid deficiency appears to be the experimental analogue of sprue (W. J. Darby, E. Jones, and H. C. Johnson, *Science* **103,** *108 (1946)*). These studies also imply that pellagra in man may be a complex deficiency state rather than a simple niacin deficiency. This point remains to be investigated directly, although there is much other evidence indicating the probability that such is the case.

EVALUATION OF THE INTRADERMAL DYE TEST

A simple means whereby the ascorbic acid status of human subjects might be estimated would be of value. Such a test was proposed in 1938 by H. Rotter (*Wien. klin. Wchnschr.* **51,** *205 (1938)*) whose intradermal dye discoloration test has been

used by various investigators. B. Portnoy and J. F. Wilkinson (*Brit. Med. J.* **1,** *328 (1938)*) and S. Suzuki (*J. Orient. Med. (Abstr. Sec.)* **30,** *267 (1939)*) maintained that a satisfactory correlation existed between this procedure and the blood ascorbic acid level, and H. Beck and F. H. Krieger (*Deutsche med. Wchnschr.* **65,** *1336 (1939)*) found this test to agree with the results obtained in ascorbic acid saturation studies. G. A. Goldsmith, D. F. Gowe, and A. T. Ogaard (*Proc. Soc. Exp. Biol. Med.* **41,** *370 (1939)*), on the other hand, concluded that no correlation existed between the plasma ascorbic acid level of fasting subjects and the intradermal dye discoloration, and I. S. Wright and E. MacLenathen (*J. Lab. Clin. Med.* **24,** *806 (1939)*) observed that the variation in the discoloration time using Rotter's method was too great to establish a normal range. These workers showed, furthermore, that discoloration times varied appreciably at different body sites.

A. H. Holland, J. C. Canniff, and M. Bruger (*J. Lab. Clin. Med.* **32,** *124 (1947)*) undertook to compare the intradermal dye discoloration time with the results of the fasting plasma ascorbic acid level, the ascorbic acid saturation test, and the capillary fragility test in 34 patients and 14 normal subjects. In each instance the tests were run consecutively in the morning after an overnight fast. The intradermal test was carried out simultaneously on both arms in accordance with the procedure described by L. B. Slobody (*Ibid.* **29,** *464 (1944)*). The average of the two tests was taken as the dye discoloration time. The capillary fragility test was performed and interpreted according to the procedure and standards outlined by I. S. Wright and A. Lilienfeld (*Arch. Int. Med.* **57,** *241 (1936)*) while the plasma ascorbic acid was determined by the method of C. J. Farmer and A. F. Abt (*Proc. Soc. Exp. Biol. Med.* **34,** *146 (1936)*). The vitamin saturation studies were carried out in accordance with the procedure of I. S. Wright and J. B. Ludden (*Med. Clin. North America* **24,** *743 (1940)*), 1 g. of ascorbic acid being injected intravenously after a twelve hour fast and urine specimens being collected at one and one-half, five, and twenty-four hours after the injection. An excretion rate of over 450 mg. of ascorbic acid was considered normal for the five hour period and over 500 mg. for the twenty-four hour saturation index.

Serious technical difficulties and inconsistencies were encountered in the dye discoloration test as well as a high degree of discrepancy when compared with the saturation test. Thus, allergic reactions such as induration and reddening at the site of the injection were occasionally noted twenty-four to ninety-six hours after the administration of the dye. In one subject the local reaction was marked and accompanied by an extensive skin eruption. Significant variations in dye discoloration times were observed when the test was performed simultaneously on both arms. Finally, some difficulty was experienced in reading the absolute end point of dye discoloration and some degree of uncertainty was always present concerning the validity of an absolute intradermal injection.

As would be expected, the capillary fragility test was found to be nonspecific. While it is admitted that increased capillary fragility can be demonstrated in subjects receiving inadequate amounts of ascorbic acid, this sign can be observed in a number of clinical conditions other than scurvy such as infectious disorders, blood dyscrasias, and hypertension. A satisfactory correlation was observed only between the fasting plasma level and the five hour urinary excretion of ascorbic acid.

PLASMA TOCOPHEROL LEVELS IN SPRUE

Since it is well established that tocopherol (vitamin E) is a dietary essential for a wide variety of species, e.g., rat, mouse, rabbit, guinea pig, dog, and chicken (S. B. Wolbach and O. A. Bessey, *Physiol. Rev.* **22,** *233 (1942)*) it is not unreasonable to anticipate that man also needs this vitamin. However, repeated attempts to show such a requirement have failed to provide satisfactory positive evidence. This may be due in part to lack of knowledge of the type of manifestations such a deficiency produces in man, or to their subtle nature. However, the tocopherols are so widely distributed in natural foods that perhaps it is rare for a diet to be so restricted and distorted as to be inadequate in these factors. It may be that tocopherol deficiency like vitamin K deficiency appears in man only under conditions where there is a failure of proper absorption from the gastrointestinal tract and, therefore, perhaps it is in this direction that information should be sought. In this connection a paper by W. J. Darby, M. E. Cherrington, and J. M. Ruffin (*Proc. Soc. Exp. Biol. Med.* **63,** *310 (1946)*) on the plasma tocopherol levels in sprue is of interest.

The plasma tocopherol levels of 20 patients with sprue in relapse or remission were studied. The authors cite examples to show that patients in relapse following withholding of pteroylglutamic acid show a gradually decreasing plasma tocopherol level which is again increased upon reinstitution of therapy. Plasma levels as low as 0.1 to 0.3 mg. per 100 ml. were found, whereas the corresponding analysis for 10 apparently normal adults was 1.06 ± 0.06 mg. per 100 ml. In 17 patients with sprue in remission following treatment with liver extract, the tocopherol levels were 0.97± 0.08 mg. per 100 ml. The decreased efficiency of absorption of the tocopherols during relapse was shown by the fact that a 600 mg. oral dose of mixed tocopherols resulted in a maximum rise of only 0.09 mg. per 100 ml. in one patient in contrast to a mean maximal rise of 0.37 mg. in 7 healthy adults after a similar dose.

Darby *et al.* (*loc. cit.*) report low values of serum vitamin E in an infant with cystic fibrosis of the pancreas and in some patients having hepatitis or cirrhosis. A. S. Minot (*J. Lab. Clin. Med.* **29,** *772 (1944)*) previously reported a low value in a child with celiac disease. These diseases are those in which poor absorption of the other fat soluble vitamins have been frequently reported, e.g., vitamin A, carotene, vitamin K, and vitamin D, and in which multiple dietary deficiencies often become a conspicuous part of the disease process.

In view of the above facts the authors believe that some of the pteroylglutamic acid compounds may be concerned with the absorption and utilization of fat soluble vitamins. However, since fat absorption in general is affected it seems unlikely that there is a specific relation to the absorption of the vitamins alone. In any event, it would seem that in seeking for tocopherol deficiency in man it might "be fruitful to study carefully active cases of steatorrhea."

PTEROYLGLUTAMIC ACID AND CONJUGATE IN MACROCYTIC ANEMIA

As a consequence of the classic investigations of W. B. Castle and associates (*Ann. Int. Med.* **7,** *2 (1933)*) most studies bearing on the etiology of the macrocytic anemias have been concerned with attempts to relate the condition under observation to deficiency of a gastric (intrinsic) or a dietary (extrinsic) factor or to some dis-

turbance in the absorption or utilization of the products of their interaction. Several investigators, however, have presented evidence that this theory cannot be applied universally. Thus it was shown that some cases of macrocytic anemia, particularly those associated with nutritional deficiencies and pregnancy, failed to respond adequately to the parenteral administration of concentrated antipernicious anemia principle but showed reversion to normal erythropoiesis when crude preparations of liver were given either by oral or parenteral routes or when autolyzed yeast was administered by mouth. As a consequence the existence of a hematopoietic substance in crude liver extract and yeast other than the antipernicious anemia factor present in purified liver extract was postulated by L. Wills and B. D. F. Evans (*Lancet* **II,** *416 (1938)*). These investigators showed that lack of this substance was responsible for cases of tropical macrocytic anemia as well as for an experimentally produced macrocytic anemia of monkeys and suggested that a similar deficiency in sprue might develop from failure of absorption. They identified the substance tentatively as a part of the vitamin B-complex and pointed out that it differed from dietary "extrinsic factor" if the hematopoietic activity of the latter, in accordance with the theory of M. B. Strauss and W. B. Castle (*New Engl. J. Med.* **207,** *55 (1932)*), was limited to a role in the formation of the liver principle.

Later, W. B. Castle together with J. Watson (*Am. J. Med. Sci.* **211,** *513 (1946)*) showed that nutritional macrocytic anemia, especially in pregnancy, may not be due to a lack of the same substance as is present in antipernicious anemia liver principle. In 3 patients there was no response to parenterally administered liver extract given in dosages effective in the treatment of Addisonian pernicious anemia but there were good hematologic and clinical results when a liver extract was given by mouth. In a fourth patient a response was obtained only when the parenterally administered dose of liver extract was increased ten times.

M. M. Wintrobe (*Am. J. Med. Sci.* **197,** *286 (1939)*) questioned the validity of attributing the hematopoietic effect of yeast exclusively to its "extrinsic factor" content, as had been assumed until that time. He demonstrated that brewers' yeast if given in large doses was effective in relieving the anemia in pernicious anemia and showed that there is a great variability in response in patients with this disease. From this work it appeared that patients with pernicious anemia may be able to utilize some substance other than the antipernicious anemia liver principle.

The discovery of the activity of folic acid in animal and in bacterial nutrition, as well as the demonstration of its effectiveness in the treatment of macrocytic anemias in man, has stimulated further studies bearing on the pathogenesis of the macrocytic anemias. With the thought in mind that in pernicious anemia there is an apparent deficiency of the vitamin, pteroylglutamic acid, which cannot in most cases be explained by dietary inadequacies, and since folic acid occurs in foods almost wholly in the conjugated form, F. H. Bethell *et al.* (*J. Lab. Clin. Med.* **32,** *3, 23 (1947)*) compared the hematopoietic responses of suitable patients with macrocytic anemia following the administration, in sequence, of the vitamin in the bound and free states. To obtain an estimate of the ability of such patients to release the vitamin from its conjugate, the urinary excretion of the free material was determined after the oral administration of the conjugate.

Pteroylglutamic acid and its hexaglutamyl conjugate were given orally to 16 patients. Three patients with pernicious anemia in relapse and one patient with anemia following gastrectomy exhibited little or no evidence of erythrocyte regeneration when given the hexaglutamyl

conjugate in a yeast concentrate containing strong conjugase inhibiting activity. When these patients received either equivalent doses of synthetic pteroylglutamic acid or the yeast concentrate after incubation with an enzyme preparation which liberated the free vitamin from its conjugated form, significant responses occurred. Two patients with pernicious anemia in relapse had definite hematopoietic responses when they were given the equivalent of 5 mg. daily of pteroylglutamic acid as the hexaglutamyl conjugate in a yeast concentrate containing relatively little enzyme inhibitor. A patient with probable nutritional macrocytic anemia showed a suboptimal response to the administration of the yeast concentrate with strong conjugase inhibiting activity.

Determinations made prior to the institution of therapy revealed that the urinary excretion of pteroylglutamic acid was lower in patients with macrocytic anemia than in normal subjects or in patients with pernicious anemia in liver extract-induced remission. In patients with pernicious anemia, postgastrectomy macrocytic anemia, and liver disease with macrocytic anemia, the urinary excretion of free vitamin after the oral ingestion of conjugate with large amounts of inhibitor was much less than the level obtained in normal subjects. One patient with probable nutritional macrocytic anemia excreted the vitamin in amounts comparable to normal subjects.

In one patient with pernicious anemia previously untreated and in another who had been receiving treatment with pteroylglutamic acid without production of complete remission, the simultaneous administration for short periods of refined liver extract by intramuscular injection and the hexaglutamyl conjugate by mouth was not followed by increased pteroylglutamic acid excretion above the levels which had been observed when the conjugate was given alone. On the other hand, the administration of conjugated vitamin to 3 patients with pernicious anemia in remission, induced by liver extract, was followed by the excretion of the free material in amounts approximately equal to those obtained in normal subjects.

The investigators concluded that patients with pernicious anemia in relapse and postgastrectomy macrocytic anemia cannot readily utilize for hematopoiesis a naturally occurring conjugate of pteroylglutamic acid although they respond promptly to administration of the free vitamin. This defect is not absolute and varies in different patients. Inability to use the conjugated vitamin appears to depend, at least in part, upon a conjugase-inhibiting substance present in natural sources of conjugate. One of the pharmacologic actions of the liver principle may be the correction of the metabolic defect in the utilization of naturally occurring conjugated forms of pteroylglutamic acid.

From this viewpoint pernicious anemia would still be a "conditioned" deficiency which depends on the inability of the individual to derive from normal diets a substance (pteroylglutamic acid) which the ordinary individual is able to obtain. Possibly in pernicious anemia liberation of the free vitamin from conjugates may be adversely affected by defective intestinal conjugase activity aggravated by the presence of the enzyme inhibiting substances. Possibly also the status of cellular enzyme systems may govern the capacity of the tissues to utilize the vitamin even after it has been successfully liberated from the conjugate and absorbed from the alimentary tract. In cases of nutritional macrocytic anemia, on the other hand, the liberation and absorption of conjugated pteroylglutamic acid in the presence of conjugase inhibitor may not be impaired.

The stomach may perhaps be concerned with the splitting of the conjugate. The intrinsic factor, although not itself capable

of converting the hexaglutamyl conjugate to the free form (A. D. Welch, R. W. Heinle, E. M. Nelson, and H. V. Nelson, *J. Biol. Chem.* **164,** *787 (1946)*) may act enzymatically as an anti-inhibitor. Supporting this suggestion are the observations of Bethell and his associates in 2 patients with macrocytic anemia following gastrectomy and in one patient who underwent subtotal gastrectomy for cancer of the stomach, in none of whom was a response to the administration of the hexaglutamyl conjugate observed.

The action of liver extract, however, is more complex than simply to relieve a conditioned nutritional deficiency of pteroylglutamic acid. This is indicated by the observation that this vitamin, administered either orally or parenterally, is not equally effective in producing remissions in all patients with pernicious anemia. Furthermore it seems to have no effect on the combined system degeneration frequently associated with this disease (C. F. Vilter, R. W. Vilter, and T. D. Spies, *J. Lab. Clin. Med.* **32,** *336 (1947)*). The variable degree of central nervous system involvement seen in patients with pernicious anemia suggests that a complex type of cellular enzyme disturbance may exist and that the action of the liver principle in restoring normal pteroylglutamic acid metabolism constitutes but one of its therapeutic effects.

ABSORPTION OF RADIOACTIVE IRON BY CHILDREN

Methods for the accurate determination of the nutritional status of children or adults with respect to iron have been limited by inadequate criteria for the evaluation of mild iron deficiencies. Present knowledge of the requirement for iron has been based on surveys of hemoglobin concentrations and of the dietary intake of iron. Few direct measurements of iron absorption and utilization have been available to supplement the limitations of the indirect data obtained from these two methods. To obtain more exact data, the absorption of radioactive iron by a large group of schoolchildren has been investigated by W. J. Darby *et al.* (*J. Nutrition* **33,** *107 (1947)*). The subjects were 188 white children, mostly between 7 and 10 years of age, who attended the second and third grades of two schools in Nashville, Tennessee. School C served a low economic area, while school R served a district of considerably higher economic status. As methods for comparing the physical well-being and the nutritional status of the children, especially in regard to their iron nutriture, the following observations were made on each child: physical examination, calculation of a seven day food intake record, and laboratory determinations of total hemoglobin, packed cell volume, serum albumin, serum vitamin A, and ascorbic acid on a sample of venous blood. Each child was given orally in a cup of lemonade 2 to 3 mg. of ferrous chloride containing Fe 59, 47 day half-life, which had been reduced with a slight excess of ascorbic acid. The cup was rinsed with a second serving of lemonade without added iron. This amount of iron was roughly comparable to that ingested in an average meal. Since the radioactive iron aliquot was given not less than an hour before or after lunch, maximum absorption was expected. Two to four weeks later a 10 ml. sample of venous blood was taken for an estimation of the radioactivity of the hemoglobin iron and for determinations of the packed cell volume and the hemoglobin concentration. From the packed cell volume and the body weight,the total red cell mass was estimated by the formula of W. M. Balfour *et al.* (*J. Exp. Med.* **76,** *15 (1942)*). The total radioactivity

of the circulating hemoglobin iron could then be estimated and expressed in terms of per cent of the administered radioiron. When this figure was used by the authors as an index of the amount of iron absorbed, it was necessary for them to assume that utilization of the absorbed radioiron had been complete. The validity of this assumption has been demonstrated for adult iron deficient animals (P. F. Hahn and co-workers, *Ibid.* **70,** *443* (*1939*)) and for both healthy and iron deficient adult human subjects (R. Dubach, C. V. Moore, and V. Minnich, *J. Lab. Clin. Med.* **31,** *1201* (*1946*)). There is no reason to suspect that the method would not give reasonably accurate results in healthy, growing children, although the completeness with which growing children utilize iron has never actually been defined.

The pronounced difference in economic status of the children attending the two schools was readily apparent from the physical examination, the calculated food intake record, and some of the laboratory examinations. The children from school R in the high economic area consumed diets which were more abundant in calories, protein, vitamins, and minerals, had greater body weights at comparable ages, and had higher levels of serum ascorbic acid and carotene than the children attending school C in the poorer district. By all tests the children in school R were well nourished. Despite the differences recorded above, the differences in mean hemoglobin concentrations by sex for the ages 7 to 10 were just on the border of statistical significance, with a trend toward lower values in the children of school C. These authors felt that the magnitude of the mean hemoglobin differences was not great enough to indicate a physiologically important distinction between the children of the two schools. The mean hemoglobin values were essentially identical with the means for children recorded in various surveys and no indication was observed of the physical signs of iron deficiency sometimes observed in adults (W. J. Darby, *J. Am. Med. Assn.* **130,** *830* (*1946*)). Therefore on the basis of hemoglobin determinations it seemed unlikely that there was any appreciable degree of iron deficiency among the children of either school.

The mean iron intake of these children was calculated from the food intake records to be 11.7 = 0.36 mg. and 10.8 = 0.19 mg., respectively, for schools R and C. These mean values approximated the Recommended Dietary Allowances of the Food and Nutrition Board (*National Research Council Reprint and Circular Series* **122** (*1945*)).

The iron absorption of these children indicated a mean uptake of 7.75 to 17.75 per cent of the test dose of 2 to 3 mg. of ferrous iron. There were no consistent differences in the absorption of radioactive iron by the children in the two schools and the mean level of approximately 13 g. of hemoglobin apparently was not limited by an iron deficiency. A remarkable increase in the mean absorption of radioactive iron was observed from the 7 to the 9 year old groups. In the 8 year old group, there was observed a significant sex difference in the average percentage of iron absorbed, with the girls absorbing significantly more than the boys. These increases in average iron absorption seemed to parallel the estimated yearly iron requirements made by C. W. Heath and A. J. Patek (*Medicine* **16,** *267* (*1937*)), and in general, the differences in iron absorption parallel the calculated yearly increments of body iron. When Heath and Patek's estimated yearly gain in circulatory and parenchymal iron was considered and the factor $\frac{100}{80}$ introduced as an allowance for storage iron, the following estimated mean daily requirements of adsorbable iron were found: for boys 7, 8, 9, and 10 years old 2.6, 2.4, 3.2, and 2.7 mg., respectively; and for girls 7, 8, 9, and 10 years old 3.0, 2.3, 2.4, and 3.8 mg., re-

spectively. If it is assumed that 50 per cent of the iron consumed in the human dietary is absorbable, the Recommended Daily Allowances of the Food and Nutrition Board would appear to be generous.

These studies seem to represent extensive experimental evidence of the actual requirement for absorbable iron by growing children who were normal in all tested criteria at the time of the experiment. In this pilot study the dose of radioactive iron given to each child was from 2 to 3 mg., an amount of absorbable iron which is roughly similar to that consumed in an average meal. In future investigations it will be of interest to determine how efficiently the normal growing organism can utilize much smaller amounts of absorbable iron. However, a completely adequate knowledge of minimum iron requirements will not be possible until accurate quantitative methods have been devised for the determination of the amount of absorbable iron in foodstuffs. In the meantime the Recommended Dietary Allowances for iron should be the goal in planning adequate diets even though these allowances may appear from these studies to be generous.

EXCRETION OF AMINO ACIDS IN URINE

H. E. Sauberlich and C. A. Baumann (*J. Biol. Chem.* **166,** *417 (1946)*) have used microbiologic assays for sixteen different amino acids because of their rapidity and convenience in studying amino acid excretion in the mouse and rat. The diets of the groups of experimental animals contained either 10 or 50 per cent of casein, 4 per cent of salts, 5 per cent of corn oil, a synthetic vitamin mixture, and glucose to make up the residue of the ration. A vitamin A source was added in suitable proportion to the corn oil. The animals were maintained on the experimental diet for at least two months before the urine was collected for analysis; the rats then weighed about 200 g. and the mice 25 g. each.

For collection of urine one rat or groups of 3 to 5 mice were placed in metabolism cages provided with an outside feeder. The urine was collected under a little toluene and after twenty-four hours the funnels were rinsed with as little distilled water as possible and the urine removed. Collection was continued for six days for each animal or group. In this way four pooled samples of urine were obtained, two equivalent to thirty mouse-days each, from mice on 10 or 50 per cent of casein respectively, and two others equivalent to forty-eight rat-days each, from rats on the two levels of protein. These samples were preserved under toluene at 0°C.

In addition, determinations were also made on twenty-four hour specimens from one rat or from groups of 3 mice, to study the variations of analytical figures within a group. The variations within groups for the percentage excreted of any amino acid on a given diet were often found to be rather wide. For instance, on a 10 per cent casein diet, from 0.4 to 1.6 per cent of the ingested leucine appeared in the urine, with a mean value of 1.0 per cent for six determinations. This was reasonably close to the figure of 1.17 per cent found for the amount of leucine in a pooled sample of urine from rats on the same diet.

The methods of analysis used by the authors have been previously reported and appear to be satisfactory. In a small number of studies of this general subject the authors and other workers have reported on the presence of certain individual amino acids measured either by chemical or microbiologic procedures in normal urine. The present paper establishes the presence in urine of all of the common amino acids

for which microbiologic assays are available, and it is presumed that glycine, alanine, and hydroxyproline are also present, although methods are not available for their determination.

Individual amino acids vary to some extent regarding the percentage of the ingested acid which is excreted, but the following general conclusions were reached. First, the amount of microbiologically available amino acids which appears in the urine is dependent on the amount ingested. It is also a general observation that the mouse excretes more free amino acid than the rat; and finally many amino acids appear in urine in a bound form, presumably in peptide linkages. Figures for the percentage of ingested amino acid excreted varied from 0.08 for aspartic acid on the 50 per cent casein diet to 1.61 for proline on the 10 per cent casein diet in rats. In mice they varied from 1.01 for aspartic acid on the 10 per cent casein diet to 5.50 for arginine on the 10 per cent casein diet.

Chemical determination by various methods of the alpha-amino nitrogen content of the urine gave higher values than those calculated from microbiologic determinations of the individual amino acids. A possible explanation for this could be the presence of d-amino acids in urine, since they would not be detected by the organisms used in the microbiologic assays, while they would be detected by the chemical assay of alpha-amino nitrogen. It has been demonstrated that certain amino acids of the d series are excreted almost quantitatively when the corresponding dl acid is fed, but of course it has not been shown that any d acid is formed when a natural protein like casein is fed.

The most important reason for the discrepancy between chemical and microbiologic results was however shown to be the presence of peptides in the urine, as was demonstrated by the increase in alpha-amino nitrogen in individual amino acids after hydrolysis. There was also an increase in the amount of microbiologically available amino acids paralleling the increase in alpha-amino nitrogen after hydrolysis. Another possibility is that urine contains inhibitors like urea which restrict the growth of the assay organisms, but this was thought to be unlikely in the present study because the dilution of the urines analyzed was so great that inhibiting effects could no longer be expected. Recoveries of added phenylalanine ranged from 93 to 107 per cent, and similar values were obtained when the estimations were made over a tenfold range of dilution, equivalent to 0.01 to 0.1 ml. of rat urine per 2 ml. of assay medium.

The terminal acids in peptides probably respond to most of the chemical procedures used, and they might also respond in certain microbiologic procedures. On the other hand it is possible that peptides such as glutanylglutamic acid and glutanyltyrosine, which do not replace glutamic acid in the growth of Lactobacillus arabinosus (J. C. Lewis and H. S. Olcott, *J. Biol. Chem.* **157,** *265 (1945)*) are more typical of amino acids in general. It is doubtful whether the method of microbiologic assay for any single amino acid has been studied adequately to determine the effects of compounds other than the one being assayed, so that doubt may also be felt of the results of microbiologic assays of a complex mixture such as urine. However, the assays undoubtedly yield at least roughly quantitative results, as shown by the parallelism observed in this study between the amounts of protein ingested and the amounts of amino acids excreted in microbiologically available form.

NUTRITIONAL REQUIREMENTS OF BRUCELLA

Chronic brucellosis (undulant fever) is a widespread debilitating disease whose diagnosis often presents difficulty because of difficulty in culturing the causative organisms. These belong to the genus Brucella. Knowledge of the nutritional requirements of these organisms would facilitate diagnosis, and perhaps eventually permit a rational approach to therapy of brucellosis. Common bacteriologic practice has been to cultivate these organisms in complex mediums of an undefined nature, such as tryptose broth.

Early investigators established that many strains of these organisms when newly isolated from diseased animals failed to grow on complex mediums unless the carbon dioxide tension was raised artificially to levels higher than occur in air. On long-continued subculture in the laboratory this high requirement for carbon dioxide is lost. Using such "acclimated" strains, S. A. Koser, B. B. Breslove, and A. Dorfman (*J. Infect. Dis.* **69,** *114 (1941)*) were able to grow Brucella in a chemically defined medium consisting of seventeen amino acids, glucose, inorganic salts, thiamine, nicotinamide, biotin, and pantothenic acid. All strains required thiamine and nicotinamide and were stimulated by pantothenic acid.

The detailed nutrition of one strain of Brucella suis has been investigated with signal success by W. G. McCullough and co-workers (*J. Bact.* **53,** *5 (1947)*), who were able to develop a chemically defined medium which supported much heavier growth than any medium previously developed and gave growth twenty-five to fifty times as heavy as any previously reported chemically defined medium. Cultures of Brucella abortus and Brucella melitensis, the other pathogens of the genus, also grew heavily in the new medium.

As a starting point, a crude medium containing a pancreatic casein digest, yeast extract, mineral salts, and glucose was used. Cultures were grown in Pyrex milk-dilution bottles and shaken continuously during growth to permit aeration, which is necessary for heavy growth of these organisms (H. N. Glassman and S. Elberg, *J. Bact.* **52,** *423 (1946)*). The optimum level of glucose was considerably higher, 1 per cent, than had previously been used. Subsequently, with 1 per cent of glucose added and the medium further modified by substitution of sulfuric acid hydrolyzed, vitamin free casein plus cystine, glycine, and vitamins for the pancreatic casein digest, the addition of manganese ion and glycerol were found very stimulatory to growth. By adding these stimulatory substances and further purifying the medium by successive stages, it was eventually found that (1) thiamine and niacin were essential for growth, and biotin and calcium pantothenate were stimulatory, especially when small inoculums were used; other vitamins were largely without effect; (2) yeast nucleic acid or any of its component pyrimidines and purines stimulated early growth but were not necessary for heavy growth with longer incubation periods; (3) of the amino acids, cystine, histidine, tyrosine, phenylalanine, and tryptophane were essential for growth, while glycine, lysine, arginine, methionine, glutamic acid, isoleucine, aspartic acid, serine, and threonine were stimulatory; and (4) magnesium salts were essential while manganese and iron salts were stimulatory. In the final mediums (chemically defined) developed, as many as 100 billion cells (100×10^9) per milliliter were obtained, as compared with only about 10 billion cells per milliliter obtained in the "tryptose" broth customarily used for cultivating these organisms. The yield of cells in the latter medium could be increased five- to tenfold merely by addition of optimal levels of glucose, thiamine, and iron salts and adequate aeration.

These results illustrate the practical utility of detailed knowledge of the nutritional requirements of an organism, not merely with respect to its qualitative requirements but also of its quantitative requirements for various nutritional essentials. They emphasize further that the nutritional requirements of pathogenic organisms are similar to those of nonpathogens which have been studied. It would be of great interest to determine what effect, if any, growth on these improved mediums had on the virulence or pathogenicity of Brucella.

POTASSIUM DEFICIENCY AND BIOTIN

S. G. Smith (*Science* **100,** *389* (*1944*)) reported that dogs maintained on purified diets containing the eight water soluble vitamins then available showed after a few weeks a high incidence of a progressive ascending paralysis. There were often several bouts of transient paralysis with spontaneous recovery before a final progressive stage was reached which resulted in death, unless the animals received therapy. Early signs were a peculiar gait, an arching of the neck, and a spasticity of the hind legs. The forelegs then became involved and eventually respiratory paralysis developed. The paralysis was regularly prevented by brewers' yeast and a water extract of a yeast. Seven attacks in 4 dogs promptly (eight to twelve hours) responded to synthetic biotin. A report of further work (S. G. Smith, *Am. J. Physiol.* **144,** *175* (*1945*)) eliminated folic acid and/or xanthopterin deficiency as a possible cause of the paralysis and seemed to exclude also the possibility that potassium deficiency was a factor, inasmuch as one dog treated with 100 mg. doses hourly for seven hours failed to respond, while a later dose of biotin was effective.

In attempts to obtain added information on the syndrome reported above W. R. Ruegamer, C. A. Elvehjem, and E. B. Hart (*Proc. Soc. Exp. Biol. Med.* **61,** *234* (*1946*)) found their dogs grew and remained well on a synthetic diet. However, when dogs were placed on a diet identical with that used by Smith, paralytic symptoms soon developed. Since this diet was found to be low in potassium, therapy with potassium chloride was tried. Dogs given 3 g. doses of potassium chloride and placed on a diet in which the salt mixture contained 160 g. of potassium chloride per 1000 g. of mixture showed an immediate improvement in symptoms and a good growth response which continued for thirty to forty days. Animals developed the paralysis while receiving biotin but responded to potassium therapy. These observations seem to indicate clearly that potassium deficiency is primarily responsible for the symptoms described. This conclusion has now been confirmed by S. G. Smith (*Ibid.* **63,** *339* (*1946*)). There does, however, seem to be an indirect effect of biotin which explains Smith's original observations. In potassium-treated dogs the reversal of the paralysis is rapid after it is once indicated. There is usually a three to six hour delay before improvement is evident, but after that a severely paralyzed dog may walk almost normally after a few minutes. A single adequate dose of potassium affords protection from paralysis for six to ten weeks; 200 mg. of potassium chloride per kilogram of body weight is an adequate curative dose, while 100 mg. per kilogram fails. Intermediate amounts of potassium have not been tried. In contrast to this dramatic and apparently complete response, biotin-treated dogs improve gradually for eight to ten hours after an initial lag period of eight hours and then relapse after a few hours. Thus, although biotin seems to

exert a temporary indirect effect it is evident that potassium is the primary deficiency. It is pointed out that serum potassium is definitely lowered during an attack of paralysis. A similar low serum potassium occurs in cases of familial periodic paralysis in man. Here also the potassium returns to normal after the attack subsides. The cause of this neuromuscular disease is unknown. The relationship between biotin and potassium which might explain the above observations is not known.

As it becomes possible to use increasingly more highly purified diets in experimental nutrition as a result of the availability of pure vitamins, it will be necessary to pay closer attention to the mineral mixtures used. It is clear that great care must be taken to distinguish between primary and secondary responses or causes.

EFFECTS OF PANTOTHENIC ACID AND RIBOFLAVIN DEFICIENCY ON WATER METABOLISM

Several workers have shown that rats deficient in pantothenic acid show pathologic changes in the adrenal cortices (*cf.* W. D. Salmon and R. W. Engel, *Proc. Soc. Exp. Biol. Med.* **45,** *621 (1940)*). L. L. Ashburn (*Pub. Health Reports* **55,** *1337 (1940)*) has suggested, on morphologic grounds, that adrenal cortical insufficiency exists in such deficient rats. However, no physiologic evidence has previously been provided to show that the adrenal changes result from, or are associated with, impairment of adrenal function. It is known, however, that administration of adrenal cortical extract, desoxycorticosterone acetate, and anterior pituitary extract fail to prevent adrenal hemorrhage in pantothenic acid deficient rats (C. W. Mushett and K. Unna, *J. Nutrition* **22,** *565 (1941)*).

Starting from the well established fact that hypofunction of the adrenal cortex in animals and man results in marked deficiencies in water diuresis and in resistance to water intoxication, R. Gaunt, M. Liling, and C. W. Mushett (*Endocrinology* **38,** *127 (1946)*) attempted to determine whether pantothenic acid deficiency results in similar changes. In addition to normal control animals, riboflavin deficient animals, which showed no lesions of the adrenal cortex, were also tested. Rats were placed on the appropriate deficient ration at the age of 3 weeks; after four to six weeks on these diets the animals were used for the experiment. Either five or thirteen doses of water (5 ml. per 100 g. of body weight) were given by stomach tube at half-hour intervals. The former dose normally does not produce intoxication; the latter does. In every case, pantothenic acid deficient animals excreted water less rapidly than normal controls and succumbed in much greater numbers from water intoxication. Normal diuresis and resistance to water intoxication were restored by administration of two doses of 1.0 mg. of calcium pantothenate by stomach tube eighteen hours and one-half hour before the test. Administration of desoxycorticosterone acetate (two doses of 1.5 mg. each, in 0.15 ml. of peanut oil, given respectively, eighteen hours and one-half hour before the test) plus 0.5 ml. of adrenal cortical extract (given with the third and sixth doses of water) completely restored normal diuresis and resistance to water intoxication in another group of pantothenic acid deficient rats. In the absence of further data, it would appear that pantothenic acid produced its effects by impairment of the mechanism by which cortical hormones are formed and secreted, and that the defects thus produced in water metabolism were remedied by supplying either

pantothenic acid or the appropriate hormones.

That this explanation is not fully correct was shown by the fact that entirely similar, though somewhat less dramatic, deviations in water metabolism from the normal controls were seen in riboflavin deficient rats. These, too, were prevented by preliminary administration of riboflavin or of cortical hormones. There are no reports which suggest impairment of adrenal function in riboflavin deficiency. Whatever the causes, it is apparent that some deficiency in water metabolism characterizes both avitaminotic states. In riboflavin deficiency, the results showed this to be most marked when symptoms of vitamin lack were severe. In pantothenic acid deficiency, however, defective water metabolism was apparent when the symptoms of vitamin deficiency were mild, i.e., when the animals, aside from reduced growth and achromotrichia, still appeared healthy. From their own results with pair-fed animals, and from experiments of others, the authors emphasize that such deficiencies in handling water, while they are apparent in various conditions, are due to specific causes, and are not characteristic of all states of general injury, debility, and weakness.

LYCOMARASMIN AND STREPOGENIN

Many microorganisms elaborate substances which can exert toxic effects on other cells. Such substances are to some extent specific in their action; those which act primarily on certain tissues of higher animals are referred to as toxins, while those which are most active against microorganisms are classed as antibiotics. An example of a third class, capable of causing injury in higher plants, is a substance produced by Fusarium lycoperseci SACC., the fungus responsible for the common disease of tomato plants known as "Fusarium wilt."

Pl. A. Plattner and N. Clauson-Kaas (*Helvet. chim. acta* **28,** *188* (*1945*)) isolated from cultures of this organism, after growth for two to four months on glucose and inorganic salts, a pure compound which accounts completely for the wilting power of the fungus. About 100 mg. of pure substance could be isolated per liter of culture filtrate, this representing a yield of only 20 per cent of the substance present. When leaves of young tomato plants, which ordinarily remain fresh for weeks in water, were immersed in solutions containing 200 mg. of the active material per liter, marked wilting occurred within three days. Low concentrations of ferric ions (0.001 per cent) increase strikingly the wilting activity of the material.

Chemical studies showed the substance to be an acid having the empirical formula $(C_9H_{15}O_7N_3)_n$. The free acid is practically insoluble in water, but the sodium salt is readily soluble. In neutral solutions the substance forms a colorless complex with ferric ions, and the authors suggest that this fact may bear a relationship to the effect of iron on the wilting activity of the compound. On mild heating in acid or alkaline mediums, or on boiling with water, the substance loses ammonia with the formation of a new compound, $(C_9H_{12}O_7N)_m$, which is devoid of wilting activity and has also lost the ability to form a complex with ferric ions.

In a later communication the same authors (*Experientia* **1,** *195* (*1945*)) reported that on complete acid hydrolysis of the active material, ammonia, aspartic acid, glycine, and a substance tentatively identified as pyruvic acid are formed. Since the molecular weight, as determined by diffusion rates, indicates that the active substance consists of 1 molecule of each of the above products, the authors proposed a pro-

visional structural formula. The name "lycomarasmin" has been suggested for the new compound.

The revelation that lycomarasmin is a peptide containing aspartic acid led D. W. Woolley (*J. Biol. Chem.* **166,** *783 (1946)*) to the hypothesis that it might exert its effect by virtue of a structural similarity to strepogenin, the substance which Woolley, in collaboration with H. Sprince, had shown to be a growth factor for a number of microorganisms as well as for mice (*Nutrition Reviews* **4,** *273 (1946)*). Although the chemical composition of strepogenin had not been worked out, the hypothesis appeared justified from the evidence at hand, which indicated that strepogenin was a peptide containing glutamic acid.

The hypothesis has indeed proved to be a fruitful one. Two new peptides, seryl-glycyl-aspartic acid and glycyl-seryl-aspartic acid, have been synthesized and found to have wilting activity approaching that of natural lycomarasmin, although they differed from the natural product in having greater solubility in water. Seryl-glycyl-glutamic acid has been synthesized and found to have strepogenin activity when tested on Lactobacillus casei. Either natural strepogenin or the synthetic glutamic peptide has been found to abolish the wilting action of lycomarasmin or the aspartic peptides. Furthermore, the latter substances have been found to inhibit the growth of L. casei, an effect which could be reversed by either strepogenin or the synthetic glutamic peptide. The antagonism between lycomarasmin and strepogenin is reported to be "competitive, at least over a limited range of concentrations."

Thus, considerable light has been shed not only on the mechanism of action of lycomarasmin but also on the chemical nature of strepogenin. However, Woolley does not regard seryl-glycyl-glutamic acid as identical with strepogenin, since the synthetic substance is much less potent than the natural product and has a molecular weight somewhat lower than the value of 300 to 500 found by diffusion studies on natural strepogenin.

To our knowledge, the work of Woolley constitutes the first demonstration that the principle of competitive inhibition is operative in the action of the natural toxins as well as in the action of the man-made growth inhibitors which have been constructed on this principle.

It also becomes apparent from the fact that the tomato wilting factor is an inhibitor of L. casei that a classification of toxins according to action on plants, animals, and microorganisms, may be rather arbitrary. In a report by E. Gaümann, O. Jaag, and R. Braun (*Experientia* **3,** *70 (1947)*), the toxic action of lycomarasmin, which is nominally a toxin for plants, and that of patulin (clavicin), which is nominally an antibiotic, were compared on some plant objects. Patulin showed considerable toxicity in all tests, whereas lycomarasmin was relatively nontoxic. It appears that the common denominator for determining the response of a cell to a toxin may lie rather in the metabolic characteristics and nutritional requirements of the cell than in its biologic classification in the plant or animal kingdom.

BACTERIAL GROWTH INHIBITION

The way in which the growth inhibition of bacteria or other unicellular organisms can be studied and used for extension of our knowledge of the reactions occurring in the protoplasm of the cell has been discussed in an earlier article (*Nutrition Re-*

views **4,** *315 (1946)*) but more recent developments appear to deserve renewed attention.

The method of reasoning employed has been termed "inhibition analysis" by J. M. Ravel and W. Shive (*J. Biol. Chem.* **166,** *407 (1946)*), who made the earlier proposal (*loc. cit.*) that the antibacterial index is a function of an inhibited enzyme system which becomes the limiting factor for the growth of the organism. It was also proposed that if the product of this enzyme system is supplied to the organism, the analogue acting as a growth inhibitor either becomes ineffective, or at higher concentrations affects another enzyme system, when a higher antibacterial index, corresponding to the second enzyme system, is obtained.

In the present investigation, the authors have extended their studies with Escherichia coli to cover its inhibition by cysteic acid, which occurred only in the absence of beta-alanine or pantothenic acid. The competitive cysteic acid-aspartic acid growth inhibition observed with E. coli was found to be a function of the enzyme system which decarboxylates aspartic acid to beta-alanine. No evidence was found that any other enzyme utilizing aspartic acid is blocked by cysteic acid, and therefore as would be expected the addition of beta-alanine or pantothenic acid to the medium supplies the limiting factor for growth.

The inhibition of the growth of Lactobacillus casei, Lactobacillus arabinosus, and Leuconostoc mesenteroides with cysteic acid is also competitive; the cysteic acid apparently competes with aspartic acid for a common enzyme which normally combines with aspartic acid as a substrate. For the organism Lactobacillus arabinosus this enzyme is not one which is concerned with the synthesis of beta-alanine. An unexpected result obtained with E. coli indicated that glutamic acid is more effective than aspartic acid in preventing the toxicity of cysteic acid, and to follow this up, the effects of alpha-keto-glutaric acid and of a mixture of alpha-keto-glutoglutaric acid and aspartic acid were investigated comparatively. There appears to be no doubt that a situation exists here in which a precursor of a metabolite (glutamic acid) exerts a greater effect in reversing the toxicity of a metabolic analogue than does the metabolite itself (aspartic acid), since the glutamic acid is three times as effective with E. coli. From the effects with hydroxyaspartic acid as well as those with alpha-keto-glutaric acid and with glutamic acid it was deduced that this greater effect of the precursor can probably be accounted for by the activity of the transaminase which is known to be present in E. coli. By the mediation of this enzyme, glutamic acid would be converted into aspartic acid and alpha-keto-glutaric acid, and it might be expected that glutamic acid would show a greater activity than aspartic acid.

By means of similar technics, J. O. Lampen and M. J. Jones (*J. Biol. Chem.* **166,** *435 (1946)*) have investigated with certain lactobacilli and enterococci the antagonism of sulfonamide inhibition by pteroylglutamic acid and related compounds. As they point out, several lines of evidence indicate an interrelation between pteroic acid derivatives (L. casei factor, folic acid, etc.) and sulfonamides in bacterial metabolism. L. J. Teply, A. E. Axelrod, and C. A. Elvehjem (*J. Pharmacol. Exp. Therap.* **77,** *207 (1943)*) found that the inhibition of Lactobacillus arabinosus by sulfapyridine was reversed by a factor in liver extract resembling folic acid, A. K. Miller (*Proc. Soc. Exp. Biol. Med.* **57,** *151 (1944)*) found that sulfanilamide can inhibit the synthesis of folic acid by E. coli, and finally R. B. Angier *et al.* (*Science* **103,** *667 (1946)*) showed that a para-aminobenzoic acid residue occurs in the pteroic acid molecule.

These authors observed that the growth of Streptococcus faecalis R and L. casei in the presence of pteroic acid derivatives and thymine is relatively insensitive to sulfonamides, although at very high sulfonamide concentrations some growth in-

hibition does occur. S. faecalis (Ralston) apparently synthesizes its own requirement of pteroylglutamic acid at a suboptimal rate, and this organism is antagonized noncompetitively by pteroylglutamic acid, pteroyltriglutamic acid, and thymine. Enterococcus strains which require preformed pteroylglutamic acid were found to be relatively insensitive to sulfonamides, but strains able to synthesize the molecule were found to be sensitive when dependent on the synthesis, although resistant in the presence of the preformed molecule.

It was concluded that the primary action of the sulfonamides under the test conditions is directed against the synthesis of pteroylglutamic acid by way of para-aminobenzoic acid, so that the effect of the sulfonamide is to produce a pteroylglutamic acid deficiency. The antagonism of this induced deficiency by the addition of the preformed molecule is thus analogous to the correction of the natural deficiency by the preformed molecule for organisms unable to synthesize pteroylglutamic acid.

DIET AND LACTATION IN RATS

The study of lactation in female rats transferred from stock to experimental diets following parturition yields results in the subsequent three week period which are qualitatively similar to those obtained in rats maintained on experimental diets from weaning or from birth (M. M. Nelson and H. M. Evans, *Arch. Biochem.* **12,** *213, 229 (1947)*). This is an important observation because it shortens the test period in lactation studies from approximately eight months to three weeks. The report of Nelson and Evans supports the conclusions that a purified diet which permits normal growth may not necessarily allow satisfactory lactation, that the storage of lactation factors before and during pregnancy may be limited, and that certain foodstuffs, notably liver, contain one or more unidentified nutrients essential for the optimum nutrition of the lactating female rat and of the suckling young. The criteria of satisfactory lactation in these experiments in which purified diets were employed were the successful weaning of 90 per cent or more of young rats weighing the same as weanlings in the stock colony and the attainment of the same moderate increase in weight in lactating females that characterized similar females on the stock diet.

The experimental ration which equaled the stock diet in the promotion of growth during an eight to nine month period after weaning but which failed to match the stock diet in so far as the above criteria of lactation are concerned had the following composition: casein 24, sucrose 64, hydrogenated vegetable oil 8, and salt mixture 4. Supplements consisted of corn oil 325 mg., alpha-tocopherol 3 mg., vitamin A 400 U. S. P. units, vitamin D 58 chick units, thiamine 0.5 mg., pyridoxine 0.5 mg., riboflavin 1.0 mg., para-aminobenzoic acid 1.0 mg., calcium pantothenate 5 mg., inositol 40 mg., and choline 100 mg. per 100 g. of food. The plan of the investigation did not permit the conclusion that each of the above supplements was actually necessary for reproduction and lactation. Reproduction was normal in both male and female rats that had been limited to this food mixture from birth. Its deficiency in lactation factors was apparent from the fact that females lost weight on the diet during the lactation period and their weanling young weighed appreciably less than controls.

Comparison of the lactation performance of female rats raised on the experimental diet with that of stock animals placed on the diet after parturition showed that the loss of weight in the female during lactation was marked in both groups although not as great in the short-term rats. Weanling

young in the short-term group were heavier than those in the long-term experiments but were below the stock control average. The proportion of young weaned was 90 per cent or more in both groups. Loss of weight of the female during lactation was the most striking evidence of dietary inadequacy. Its occurrence within the three week period in rats previously fed the stock diet argues against extensive storage of the factors concerned. However, the fact that lactation was better in these animals than in the group raised on the same experimental diet suggests that limited storage did occur. It is possible that the stock diet may not have been rich enough in lactation factors to have permitted more significant storage.

An increase in the casein level from 24 to 64 per cent brought about slight improvement in the long-term experiments but had no effect in the short-term lactation trials. The effect of increasing the fat level from 8 to 48 per cent was highly damaging to lactation in both groups. This was true even if the vitamin levels were doubled and hence was not the result of diminished vitamin intake due to the smaller amount of food ingested. A similar control of the effect of a diminished protein intake was not included in the experiments and it is possible that the deleterious effect of the high fat diet was due to decreased protein intake. If the rats on the high fat diet consumed the same number of calories as those on the low fat ration, the protein intake would have been decreased by one-third.

Definite evidence that the subnormal lactation observed on the purified diet was due to a readily preventable dietary inadequacy was afforded by the fact that lactation was practically normal if a commercial liver eluate powder was added to the diet. Less marked benefit resulted from additions of rice bran extract, yeast, and a commercial liver extract. Biotin was ineffective. The potent liver eluate powder was rich in folic acid but preliminary tests failed to demonstrate that this compound was involved (M. M. Nelson, F. van Nouhuys, and H. M. Evans, *Proc. Soc. Exp. Biol. Med.* **61,** *74* (*1946*)).

It is of interest in this connection that almost complete lactation failure occurs in rats on an all plant ration composed of yellow corn, soybean meal, alfalfa meal, and minerals (R. R. Spitzer and P. H. Phillips, *J. Nutrition* **32,** *631* (*1946*)). Storage of dietary essentials necessary for reproduction and lactation was indicated in these experiments. Improvement resulted if the ration was supplemented with additional alfalfa meal, liver powder, casein plus choline, or fish meal.

There is abundant evidence for the existence of dietary essentials required for normal lactation (*Nutrition Reviews* **1,** *197* (*1943*); **2,** *136* (*1944*); **3,** *343* (*1945*)). The observations of Nelson and Evans confirm and extend additional ones by L. J. Vinson and L. R. Cerecedo (*Arch. Biochem.* **3,** *389* (*1944*)) and by L. R. Richardson and A. G. Hogan (*Federation Proceedings* **4,** *161* (*1945*)). The demonstration of the usefulness of the short-term procedure in lactation studies in rats is a valuable contribution which should facilitate the identification of the nutrients required by the female to prevent loss of weight during lactation. It is not apparent whether the observed difficulties in lactation are concerned with the production of sufficient milk or whether a specific component of milk is involved. It may be that the loss of weight in the female and the failure of the young to show an optimal rate of growth during the first three weeks of life reflect the unsuccessful attempt to provide a missing factor at the expense of maternal tissues. The fact that weanling rats do grow at an optimal rate on the diet which is partially inadequate for lactation indicates that the missing factor or factors is dispensable or required in smaller amounts after rats reach 3 weeks of age.

NOTES

Destruction of Carotene in Alfalfa

The destruction of carotene in alfalfa has been discussed in this journal (*Nutrition Reviews* **1,** *153 (1943)*), although not for over four years, and the related question of the loss of carotene during storage of dehydrated vegetables has also received attention (*Ibid.* **2,** *78 (1944)*). H. L. Mitchell and H. H. King reported a study on the effect of dehydration on the enzymic destruction of carotene in alfalfa (*J. Biol. Chem.* **166,** *477 (1946)*).

Although doubt had earlier been expressed that enzymes are responsible for the destruction of carotene during the curing process, H. L. Mitchell and S. M. Hauge (*J. Biol. Chem.* **163,** *7 (1946)*) appear to have demonstrated beyond doubt that lipoxidase is responsible for a considerable part of the destruction of carotene in alfalfa as it cures in the field. There have been no reports of the carotene-destroying ability of peroxidase, which is also present in alfalfa. In a further study the same authors have investigated the question of whether these two oxidative enzymes are involved in the destruction of carotene that occurs during the storage of dehydrated alfalfa meal.

Alfalfa for the experiments was harvested in early bloom and part of the fresh material was blanched with steam at atmospheric pressure for ten minutes. The blanched and unblanched materials were then dehydrated in a pilot plant dehydrator and were ground to pass a 1 mm. screen and stored at −23°C. until used. The dehydrator in use had the hot gases at the entrance tunnel at a temperature of about 550°C., with a temperature at the exit of 150°C. to resemble the conditions in using many commercial dehydrators.

It was first demonstrated that the lipoxidase of alfalfa was inactivated during dehydration but that the peroxidase is only inactivated if the alfalfa is blanched before dehydration. The peroxidase, whether in aqueous extracts or in the meal, did not catalyze the oxidation of carotene at all. No regeneration of either lipoxidase or peroxidase activity occurred during the storage of alfalfa meal for a period of two months.

Since it was also found that blanching the alfalfa before dehydration did not lead to any greater retention of the carotene in it during storage, it was concluded that carotene destruction during storage, unlike that which occurs during the curing process, is not enzymic in nature. The loss is serious, amounting to almost 45 per cent in two months, and occurring almost equally in alfalfa meal which is or is not blanched before dehydration.

A New Metabolite of Niacin

Only a small percentage of ingested niacin reappears in the urine. Instead, a variety of derivatives of this vitamin are excreted, viz., nicotinylglycine (nicotinuric acid), N′-methylnicotinic acid (trigonelline), and N′-methylnicotinamide (F_2), which last usually predominates. However, even these several derivatives account for at most only 20 to 40 per cent of fed niacin. W. E. Knox and W. I. Grossman (*J. Biol. Chem.* **166,** *391 (1946)*), have reported another derivative of niacin which is excreted in the urine when nicotinamide is fed. The discovery of this material was unusual. W. E. Knox (*Ibid.* **163,** *699 (1946)*), in studying the destruction of quinine in the body, had found an enzyme which could oxidize quinine as well as a number of other heterocyclic compounds containing nitrogen in the ring. The oxidation occurred in each case at a carbon of the ring adjacent to the nitrogen. "The only physiological compounds found to be oxidized by this enzyme were those related to nicotinic acid. N′-methylnicotinamide was the most rapidly oxidized." The product of this oxidation was N′-methyl-6-pyridone-3-carboxylamide, i.e., F_2 with

an oxygen attached to the sixth position of the ring. Following these *in vitro* studies, 600 to 900 mg. of nicotinamide were fed to human subjects and an average of 100 mg. of the pyridone was isolated from the urine, thereby proving that the same oxidation occurs *in vivo*. Data have not been reported on the amounts of the pyridone normally present in the urine, but this compound promises to be at least as significant as F_2. If so, it will be possible to account for a substantial proportion of metabolized niacin.

Diet and Liver Disease

The relationships between dietary factors and liver disease, both experimental and naturally occurring, are discussed in a review by L. J. Witts (*Brit. Med. J.* **1**, *1*, *45* (*1947*)).

When one considers the complexity of the metabolic processes in the liver, it is not surprising that many dietary factors can affect reactions of the liver. Because of the complexity of dietary factors which may affect the liver, Witts arrives at the principle that "It therefore seems unlikely that fortification with individual principles will be as cheap or as effective as a general improvement of the diet in the prevention of hepatic diseases. Our knowledge is more likely to be useful in the selection of those natural foods which can be most expeditiously used to increase the biological value of the diet."

New British Nutrition Journal

NUTRITION: DIETETICS: CATERING is the name of a new independent quarterly review incorporating official news of the British Dietetic Association and of the Food Education Society. The first issue of this journal was published in the spring of 1947. Among other things it contains a foreword written by Sir Jack Drummond and an interesting article by Professor J. R. Marrack entitled "Food or Nutrients." For subscriptions out of the United Kingdom a charge of 12s.6d. is made and subscriptions should be sent directly to the publishers, Newman Books Ltd., 356–366 Oxford Street, London, W.1.

Canadian Nutrition Notes

The Nutrition Division of the Department of National Health and Welfare, Ottawa, Canada, publishes a monthly bulletin entitled "Canadian Nutrition Notes," which deals with events of current interest, particularly in applied nutrition activities.

Recent Nutrition Books

Experiences with Folic Acid. Tom D. Spies. The Year Book Publishers, Inc., 304 South Dearborn Street, Chicago, Ill. 1947. Pp. 105.

Feeds of the World. Their Digestibility and Composition. B. H. Schneider. Agricultural Experiment Station, West Virginia University, Morgantown, W. Va. Pp. 299.

Folic Acid (booklet). By 30 contributors; W. H. Peterson, *et al.* Annals of the New York Academy of Sciences, Vol. XLVIII, Art. 5, pages 255–350, November 30, 1946. Published by The New York Academy of Sciences, Central Park West at Seventy-ninth Street, New York, N. Y.

Foods: Their Values and Management. Henry C. Sherman. Columbia University Press, Morningside Heights, N. Y. 1946. Pp. 220.

Report of the FAO Mission for Greece. The Food and Agriculture Organization of the United Nations, Washington, D. C. 1947. Pp. 173.

Table of Food Values Recommended for Use in Canada. By the Nutrition Division, Department of National Health and Welfare, Ottawa, Canada. 1946. Published under the authority of the Hon. Brooke Claxton, K. C., M. P., Minister of National Health and Welfare. Pp. 183.

The 1946 Year Book of Endocrinology, Metabolism, and Nutrition. The Year Book Publishers, Inc., 304 S. Dearborn St., Chicago, Ill. 1947. Pp. 573.

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

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